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TURNOVER OF HUMAN AND MONKEY PLASMA KININOGENS IN RHESUS MONKEY--ETC(U)
1977 T YAMADA, D A WING, J V PIERCE

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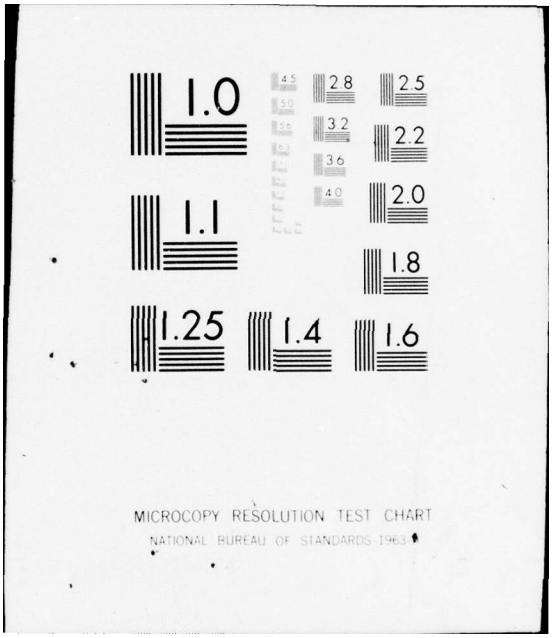
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REPORT DOCUMENTATION PAGE		READ INSTRUCTIONS BEFORE COMPLETING FORM
1. REPORT NUMBER	2. GOVT ACCESSION NO.	3. RECIPIENT'S CATALOG NUMBER
4. TITLE (and Subtitle) Turnover of human and monkey plasma kininogens in rhesus monkeys.		5. TYPE OF REPORT & PERIOD COVERED Interim 6. PERFORMING ORG. REPORT NUMBER
7. AUTHOR(s) Tadataka Yamada, David A. Wing, Jack V. Pierce, and George W. Pettit		8. CONTRACT OR GRANT NUMBER(s)
9. PERFORMING ORGANIZATION NAME AND ADDRESS U.S. Army Medical Research Institute of Infectious Diseases, Ft Detrick Frederick, MD 21701		10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS SGRD-UIP-A 62776A 3M762776 A841-00-037
11. CONTROLLING OFFICE NAME AND ADDRESS U.S. Army Medical Research and Development Command, Office of The Surgeon General Department of the Army, Washington, DC 20314		12. REPORT DATE 1977 13. NUMBER OF PAGES 33 pages plus 4 graphs
14. MONITORING AGENCY NAME & ADDRESS (if different from Controlling Office)		15. SECURITY CLASS. (of this report) UNCLASSIFIED 15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT (of this Report)

Approved for public release; distribution unlimited.

17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)

18. SUPPLEMENTARY NOTES

Reprints bearing assigned AD number will be forwarded upon receipt. To be submitted for publication in the Journal of Clinical Investigation.

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

Kininogen, turnover, monkey, protein metabolism.

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

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of high molecular weight kininogen (25.95 ± 1.60 h) (mean ± SEM) and that of low molecular weight kininogen (18.94 ± 1.93 h) was only marginally significant ($P < 0.05$). In contrast, a highly significant ($P < 0.001$) difference in their mean catabolic rates (1.12 ± 0.08 day^{-1} for high molecular weight kininogen versus 2.07 ± 0.09 day^{-1} for low molecular weight kininogen) was observed. These differences between the two kininogens were attributed to differences in their distribution between the intra- and extravascular pools. Studies of kininogen turnover will be useful in elucidating the in vivo functions of the various kininogens in health as well as during clinical illness.

Turnover of Human and Monkey Plasma Kininogens in Rhesus Monkeys

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Running Head: Turnover of Plasma Kininogens

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This work was presented in part at the VIth International Congress on Thrombosis and Haemostasis, Philadelphia, Pa., 1 July 1977.

In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals," as promulgated by the Committee on the Revision of the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Research Council. The facilities are fully accredited by the American Association for Accreditation of Laboratory Animal Care.

The views of the authors do not purport to reflect the positions of the Department of the Army or the Department of Defense.

ABSTRACT

The normal metabolic turnover of plasma kininogens was studied by measuring disappearance of intravenously administered radiolabeled human and monkey plasma kininogens from the circulation of healthy adult rhesus monkeys. Curves obtained by plotting log of radioactivity against time could be expressed as double exponential equations, with the first term representing diffusion and the second term catabolism. No significant difference between the turnovers of human and monkey kininogens was observed. The difference between the $t_{1/2}$ of high molecular weight kininogen (25.95 $\pm$ 1.60 h) (mean $\pm$ SEM) and that of low molecular weight kininogen (18.94 $\pm$ 1.93 h) was only marginally significant ($P < 0.05$). In contrast, a highly significant ($P < 0.001$) difference in their mean catabolic rates (1.12 $\pm$ 0.08 day^{-1} for high molecular weight kininogen versus 2.07 $\pm$ 0.09 day^{-1} for low molecular weight kininogen) was observed. These differences between the two kininogens were attributed to differences in their distribution between the intra- and extravascular pools. Studies of kininogen turnover will be useful in elucidating the in vivo functions of the various kininogens in health as well as during clinical illness.

INTRODUCTION

The kinins are a group of polypeptides known primarily for their potent vasodilatory actions in addition to their ability to cause increased capillary permeability and leukocyte chemotaxis (1, 2). They are generated in plasma through enzymatic cleavage of a precursor protein, kininogen, by plasma kallikrein (3). Plasma kallikrein is formed from its precursor, prekallikrein, by the action of activated Hageman factor (blood coagulation factor XII) (4). Because of their profound physiologic actions, the kinins have been implicated in the pathophysiology of a wide variety of clinical disorders including carcinoid syndrome (5), hereditary angioedema (6), dumping syndrome (7), Gram-negative septicemia (8), and disseminated intravascular coagulation (9). Documentation of the involvement of the kinin system in these various disorders, however, has been limited to the observation of elevated plasma kinin and kallikrein activities and depletion of their precursor proteins, kininogen and prekallikrein. Such observations only imply kinin system activation. No data regarding the specific kinetics of kinin system proteins in these disorders are available.

Recent advances in the isolation and purification of plasma kininogens (10) have enabled us to examine the normal turnover of these proteins. By intravenous injection into rhesus monkeys of radiolabeled purified human and monkey

kininogens and subsequent measurement of their disappearance, we have endeavored to characterize the kinetics of these proteins in plasma. We hope to use this information to study kininogen turnover in various disease states in order to clarify the role of the kinin system, if any, in their pathogenesis.

METHODS

Isolation of kininogens. Kininogens were purified using the affinity chromatography method of Pierce and Guimarães (10) as outlined in Table I. Two liters of either human or monkey blood were drawn into plastic receptacles containing 1 part 3.8% sodium citrate, 0.1% hexadimethrine bromide (Aldrich Chemical Co., Inc., Milwaukee, Wis.) and 0.01 M benzamidine chloride (Calbiochem, San Diego, Cal.), to 9 parts whole blood. The samples were centrifuged at 25°C for 10 min at 2000 g and the plasma supernatant (520 ml) was chromatographed on a 2.6 x 40.0 cm column of L-lysine coupled to Sepharose 4B (Pharmacia Fine Chemicals, Inc., Piscataway, N.J.) prepared as described by Deutsch and Mertz (11). The plasminogen-free plasma (580 ml) thus obtained was stirred into 4 liters of a solution containing 50 g dry weight of DEAE-cellulose (DE-23, Whatman Chemicals, Division of W. and R. Balston, Maidstone, Kent, England), 0.01% hexadimethrine, and 1 mM benzamidine and titrated to pH 6.0 by the addition of 5 N HCl. The resulting slurry was stirred vigorously for 2 h, allowed to settle for 30 min, decanted onto a coarse fritted Buchner funnel, and washed with 5 liters of 0.01% hexadimethrine - 1 mM benzamidine solution. The washed adsorbent was suspended with 67 ml of 3.0 M Tris-HCl buffer, pH 6.0, and enough 0.01% hexadimethrine - 1 mM benzamidine to give a final volume of 250 ml. After stirring for 1 h, the suspension was filtered in a coarse

fritted Buchner funnel and washed with 500 ml of 0.4 M Tris HCl, 0.01% hexadimethrine, 1 mM benzamidine buffer, pH 6.0. The eluate, called prep A, was adjusted to pH 7.2 and applied at 25°C to an affinity column (3.1 x 13.9 cm) prepared by coupling purified sheep anti-human low molecular weight kininogen to cyanogen bromide-activated Bio-gel A-50m (Bio-Rad Laboratories, Richmond, Cal.) by the method of March et al. (12). The column was washed with 0.4 M Tris-HCl, 0.01% hexadimethrine, 1 mM benzamidine, pH 6.0, and eluted with 8 M guanidine HCl. The purified kininogen thus obtained, called prep B, was dialyzed against 0.05 M phosphate buffer, pH 6.0 for 24 h at 25°C and chromatographed on a DEAE-cellulose column (0.9 x 30.0 cm). Four main A_{280} peaks coincident with peaks of kininogen activity, called B1, B2, B3, and B4, were eluted using a linear phosphate buffer gradient, 0.05 - 0.30 M, pH 6.0. Essentially no difference was observed between human and monkey kininogens in the number or position of the protein peaks. Approximately 48% of the kininogen activity of the initial plasma was recovered in the total activities of B1 - B4. Gel filtration of each of the peaks with Bio-gel A-0.5m revealed separate kininogen subfractions, called α, β, and γ, corresponding to proteins of molecular weight 80,000, 160,000, and 225,000, respectively. Whereas B1, B2, and B3 consisted mainly of the α protein with some β and no γ proteins, B4 consisted mainly of the γprotein

with some α and β proteins. Hence, for the purposes of our study, we will refer to B4 as high molecular weight (HMW)[1] kininogen and a pooled sample consisting of B1, B2 and B3 as low molecular weight (LMW) kininogen, unless the α, β, or γ speciation is otherwise noted.

Sheep antisera to human LMW kininogens (kininogens I and II) were prepared by the method of Pierce and Webster (13). The antisera were purified by immunoprecipitation followed by chromatography on hydroxyapatite in 8 M urea. A reaction of identity was obtained by Ouchterlony double diffusion with human and monkey HMW and LMW kininogens against the purified antiserum.

Kininogen activity was measured by bioassay on guinea pig ileum by the direct method of Prado et al. (14), modified by Webster and Prado (15) and Pierce and Guimarães (10). A 3-cm segment of ileum was suspended in 5 ml of Tyrode solution at 32°. An amount of kininogen sample estimated to release 30 ng of lysyl-bradykinin (kallidin) was added to the bath followed by 10 μl of partially purified human urinary kallikrein (10 tosyl L-arginine methyl ester units/ml) prepared by the method of Pierce and Guimarães (10). The magnitude of isotonic contraction of ileum, loaded with 1 g

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[1] Abbreviations used in this text: HMW = high molecular weight, LMW = low molecular weight.

of tension, caused by the kallidin released from kininogen was measured with a displacement transducer (Narco Bio-Systems, Inc., Houston, Texas). Results were quantified using a standard curve of ileal contractions elicited by known quantities of a standard solution of kallidin (Schwarz/Mann, Orangeburg, N.Y.).

Iodination of kininogens. Proteins for kinetic studies were radiolabeled with ^{125}I using the microdiffusion method of Gruber and Wright (16). A 50-ml Ehrlenmeyer flask was modified to contain an inner chamber by gluing a 1.3 x 2.3 cm plastic vial to its bottom. One milliliter of a protein solution at an approximate concentration of 1 mg/ml was placed in the outer chamber of the flask and 0.2 ml of 0.002 M KI and 1 mCi of $Na[^{125}I]$ (50 mCi/ml, New England Nuclear, Boston, Mass.) were placed into the inner chamber. The flask was sealed with a rubber skirt-type vaccine stopper and 0.2 ml of a 1:20 dilution of a stock acid-dichromate solution (0.27 M $Na_2Cr_2O_7$ in 36 N H_2SO_4) was added to the inner chamber with a syringe through a 4-inch 20-gauge needle. The flask was rotated gently at 25°C so that no mixing between the two chambers occurred. After 1 h, the radiolabeled protein was removed with a syringe through a 4-inch needle and separated from unreacted iodine by gel filtration on a 1.5 x 5.5 cm column of Sephadex G-25 (Pharmacia Fine Chemicals, Inc.). Efficiency of labeling using this technique was generally low (4.6 - 9.7%) but the kininogens

retained essentially all of their biologic activity (96.7 - 99.2%) after iodination.

Animal studies. Adult male rhesus monkeys (Macaca mulatta) weighing 3.5 - 6.0 kg were used. Twenty-four hours prior to injection of radiolabeled kininogen, each monkey was administered 1 ml of a saturated solution of KI orally; for the duration of the study, all fluid intake was limited to a solution containing 0.02% NaI. During the initial injection of kininogen, and for each subsequent blood sampling, the monkeys were anesthetized with ketamine-HCl (10 mg/kg, i.m.). A preparation of iodinated kininogen, in an amount not exceeding 5% of the calculated total blood content of the kininogen species injected, was administered to each animal via saphenous vein injection. Two minutes after injection, a 1.8-ml blood sample was withdrawn from a femoral vein into a siliconized glass 2-ml Vacutainer tube (Becton-Dickinson Co., Rutherford, N.J.) containing 0.2 ml of 3.8% sodium citrate, 0.1% hexadimethrine bromide, 0.01 M benzamidine, and this was considered the 0 time sample. Subsequent samples were withdrawn at 1, 2, 3, 4, 6, 8, 12, 16, 20, 24, 28, 32, 36, 40, 44, and 48 h following injection of radiolabeled kininogen.

From each 2-ml sample, 0.4 ml of whole blood was removed and stored at -70°C. The remaining blood was centrifuged at 25°C for 10 min at 2000 g and 0.4 ml of plasma supernatant was

removed and stored at -70°C. A second 0.4 ml-aliquot of plasma was mixed with 0.4 ml of 0.2 M Tris-acetate, 0.01 M EDTA buffer, pH 6.0, and 0.1 ml of antibody against human LMW kininogen. The mixture was stored at 4°C for 16 h, then centrifuged at 2000 g for 10 min at 4°C. The resulting supernatant, and three subsequent washes of the precipitate with 1 ml of 0.2 M Tris, 0.1 M EDTA buffer, pH 6.4 were poured over a 0.22-μ Millipore filter (Millipore Corporation, Bedford, Mass.). The filter and the remaining precipitate were placed into a single tube and stored at -70°C. At the end of each 48-h study, all the stored preparations were counted in a gamma counter (Model 1185, Searle Analytic, Inc., Des Plains, Ill.).

The injection schedule of radiolabeled kininogens was as follows: 6 monkeys received human prep B, 6 received human HMW kininogen, 4 received human LMW kininogen, 4 received human B3α[2], 4 received human B4γ, 4 received monkey HMW kininogen, and 4 received monkey LMW kininogen.

Data analysis. Turnover of kininogen was assessed using the plasma slope method described in the multiple pipeline model for interstitial albumin distribution by Reeve and Bailey (17). The radioactivity of each sample withdrawn from the monkeys, expressed as a percentage of the radioactivity of the initial

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[2]The A_{280} peak B3 actually consisted of two subpeaks called B3.1 and B3.2. The B3α used for our studies was B3.2α.

(time 0) sample for each monkey, was plotted on a logarithmic scale against time after injection of radiolabeled kininogen. In each case, the disappearance curve of injected kininogen from plasma could be expressed as a double exponential equation, $Y = C_1e^{-at} + C_2e^{-bt}$, where Y represents % of initial counts per minute, t represents time, C_1 and C_2 represent the constants of the two exponentials, and a and b are the rate constants. The equation that best fits the data for each test was calculated by linear regression analysis using the Simulation Analysis and Modeling (SAAM) program of Berman and Weiss (18) in a UNIVAC 1108 computer at the National Bureau of Standards. Correlation coefficients for the multiple regressions were calculated using the method described by Dixon and Massey (19).

In the double exponential model for fibrinogen turnover, the first, rapid, exponential is hypothesized to represent initial diffusion of labeled protein from the intra- to the extravascular pool. The second, slower, exponential is thought to represent disappearance of fibrinogen from the intravascular pool as a result of catabolism. Assuming a similar hypothesis for kininogen, we used the following equations as derived by Regoeczi (20) to calculate mean catabolic rate (Fc), representing the fraction of intravascular injected labeled protein catabolized in 24 h, capillary transfer rate (Fi, e), representing the fraction of intravascular protein transferred to the extravascular pool in 24 h,

return flow (Fe, i), representing the fraction of extravascular protein returned to the intravascular pool in 24 h, and pool ratio (ev/iv), representing the ratio between extra- and intravascular pool size:

$$Fc = \frac{1}{C_1/a + C_2/b} \times 24 \text{ day}^{-1}$$

$$Fi,e = (C_1 a + C_2 b) \times 24 - Fc \text{ day}^{-1}$$

$$Fe,i = (a + b) \times 24 - (Fc + Fi,e) \text{ day}^{-1}$$

$$ev/iv = \frac{Fi,e}{Fe,i}$$

C_1 and C_2, as before, represent the constants for the two exponentials and a and b represent the rate constants. Biologic half-life ($t_{1/2}$) was calculated using the standard equation:

$$t_{1/2} = \frac{\ln 2}{b}$$

The rate constant of the second exponential (b) which expresses the rate of catabolism was used to obtain $t_{1/2}$. All statistical comparisons of data were made using Students t-test.

RESULTS

The disappearance curves of radiolabeled human kininogens from plasma of rhesus monkeys could be expressed as double exponential equations (Fig. 1). Like fibrinogen (21, 22), kininogen had a high metabolic rate and first-order diffusion and catabolic characteristics. HMW and LMW kininogens had similar configurations for their turnover curves, but considerable differences in their kinetics existed. As expected, prep B, a mixture of HMW and LMW kininogens appeared to share characteristics of both forms of kininogens. A comparison of kinetic parameters between HMW and LMW kininogens is depicted in Table II. Plasma half-life of HMW kininogen was 25.95 $\pm$ 1.60 h (mean $\pm$ SE) while that of LMW kininogen was 18.94 $\pm$ 1.93 h, a difference that was only marginally significant ($P < 0.05$). On the other hand, a highly significant difference ($P < 0.001$) existed between their mean catabolic rates. Since plasma half-life is a measure of catabolism of only the kininogen in the intravascular pool, and mean catabolic rate is dependent upon both intra- and extravascular pools of kininogen, it follows that the observed differences between HMW and LMW kininogens result from differences either in intravascular-extravascular flux or in pool size. A significant difference in pool ratios existed, with HMW kininogen being partitioned primarily into the intravascular pool and LMW kininogen being more equally distributed between the two pools. Although

capillary transfer rate of HMW kininogen was slower than that of LMW kininogen, the difference was not significant. No significant difference was found in return flow for the two kininogens.

The adequacy of the model for describing the system by using double exponential equations was tested by two methods. First, the sum of the constants for the two exponentials ($C_1 + C_2$) of each of the disappearance curves was measured. Theoretically, this value should approach 100% if there are no additional exponentials required for proper fit of the disappearance curves to the calculated equations. The results shown in Table III indicate that two exponentials are all that are required to provide a good fit for the turnover curves. The second method used to evaluate adequacy of fit was simple calculation of correlation coefficients for multiple regressions. Correlation (r) values for the double exponentials were in excess of 0.9900 for all of the studies (Table IV). Equally notable was the absence of any improvement in fit of the curves to triple exponential equations.

Measurement of turnover by following disappearance of plasma radioactivity was compared with disappearance of whole blood radioactivity and antibody-precipitated kininogen antigen radioactivity. Results are shown in Fig. 2. Turnover curves obtained by measuring whole blood and immunoprecipitated kininogen radioactivity were not significantly different from plasma studies, nor were they significantly different from each

other. This was true for all forms of kininogen tested.

Since so-called HMW kininogen and LMW kininogen are each mixtures of kininogens of various molecular weights, their turnover curves were compared to those of the pure 80,000 and 225,000 molecular weight proteins, B3α and B4γ. As shown in Fig. 3, the turnover curves for B3α and B4γ were not significantly different from their counterparts of lesser purity, indicating that the heterogeneous composition of our HMW and LMW kininogen preparations did not alter the basic kinetic characteristics of their respective major components.

The data discussed have referred exclusively to turnover of human kininogens. Because of the phylogenetic similarity between monkeys and humans, and because of the apparent identity in biochemical and antigenic characteristics of their kininogens, we assumed that human kininogens would have similar turnover kinetics compared to monkey kininogens when studied in rhesus monkeys. The accuracy of this assumption was tested and our results are depicted on Figure 4. The disappearance curves for radiolabeled monkey HMW and LMW kininogens fell within the 95% confidence limits of the curves for their corresponding human kininogens. While there was no difference in their plasma half-lives, mean catabolic rate of monkey LMW kininogen, 1.10 ± 0.14 day^{-1} (mean $\pm$ SE) was significantly lower ($P < 0.001$) than that of human LMW kininogen, 2.07 ± 0.09 day^{-1}. There were no

significant differences in capillary transfer rate or in return flow to explain the lower catabolic rate, but the pool ratio for monkey LMW kininogen, 0.64 $\pm$ 0.03, was significantly smaller ($P < 0.01$) than that for human LMW kininogen, 1.052 $\pm$ 0.068.

DISCUSSION

We have endeavored to characterize the normal turnover of kininogens by measuring their disappearance from the plasma of rhesus monkeys following bolus injection. The relatively rapid metabolic rate observed for kininogens indicated that kinetic analysis by the plasma slope method would be appropriate for the study of their turnover. Because terminal hydrolysis products of radioiodinated proteins are excreted at finite rates, methods of protein turnover analysis involving measurement of urine radioactivity (23) can be inaccurate in the case of rapidly metabolized proteins (22). An inevitable lag period between catabolism and excretion exists for any protein; this lag period is short in the case of slowly metabolized proteins, but can be significant in rapidly metabolized proteins and can result in a disequilibrium between catabolism and excretion. Such circumstances could create the erroneous appearance of a site of extravascular protein sequestration prior to catabolism. In addition to theoretical reasons for avoiding urine radioactivity measurements, the technical problem of obtaining accurate urine samples from monkeys without surgical intervention was virtually insurmountable.

Our results indicated that turnover of plasma kininogens conformed to double exponential equations. In measuring turnover by the plasma slope method, we assumed a simple system consisting of two pools: the intravascular pool into

which radiolabeled kininogen was injected, and a single extravascular pool into which the injected kininogen diffused, or was catabolized. The "pools" did not represent specific anatomic compartments but were pools only in a kinetic sense, since we did not attempt complicated multicompartmental modeling analysis during our present study. According to our assumption, kininogen could exit from the intravascular pool by only two processes, diffusion or catabolism. If our double exponential hypothesis were correct, the sum of the constants for the fraction of injected protein that diffuses (C_1) and the fraction that is catabolized (C_2) should approximate 100%. As our data indicated, the average values for $C_1 + C_2$ were close to the predicted value, suggesting that there were no overlooked components to the equation describing kininogen turnover in plasma. We confirmed this observation by testing the fit of our data to triple exponentials and observed no notable improvement in correlation coefficient. These results are in contradistinction to those observed in studies of fibrinogen turnover which, although said to conform to double exponential equations, indicate the presence of a small (2-3%) third component in their turnover curves (22).

Analysis of our kininogen turnover curves was simplified and accuracy was improved considerably by utilizing the SAAM computer program. Without the program, the constants for the double exponential equations would have had to be estimated by graphic means. By extrapolation of the terminal portion of the

turnover curves to time 0, the y-intercept (C_2) and slope (-b) of the second exponential could be obtained, and by subtraction of the second exponential from the total curve, the constants (C_1 and -a) for the first exponential could be calculated. Using the SAAM program, we were able to perform repeated iterations of the data to obtain constants for the curve that provided the best fit in a relatively short time. In addition, we were able to test the fit of our data to more than two exponentials without difficulty.

To follow the disappearance of radiolabeled kininogen from the intravascular pool, we compared measurements of whole blood, plasma, and antibody-precipitated kininogen radioactivities. There were no differences in turnover curves obtained using any of the three methods of analysis, although clearly the last method was the most specific. We were not able to measure the radioactivity of functionally active kininogen as opposed to kininogen antigen. In studies of fibrinogen turnover, this problem is not encountered because plasma samples can be clotted and the radioactivity of the fibrin contained within the clots counted. However, fibrinogen turnover curves obtained by measuring plasma radioactivity were nearly identical to those obtained by measuring radioactivity of clottable protein (24). While we cannot exclude the possiblity that kininogen may continue to circulate in the intravascular pool as measurable antigen even after partial catabolism, it is unlikely, since a common pathway

for diffusion and catabolism is suggested by the observation that the catabolic pattern for kininogen was superimposed by a similar kinetic for its diffusion.

The differences we observed between the kinetics of HMW and LMW kininogens cannot be explained simply on the basis of molecular size since albumin, with a molecular weight less than that of LMW kininogen, has a half-life nearly four times as long (19). The differences in mean catabolic rate of the two kininogens were attributable primarily to differences in their pool ratios. It is tempting to speculate about the functions of the two kininogens in relation to their pool distribution, but we have little data with which to support such conjectures. However, the importance of HMW kininogen in the coagulation process has been well documented (25-27), although the specific in vivo functions of the various kininogens are otherwise unknown. In addition, Pierce and Guimarães (10) have corroborated earlier observations by Jacobsen and Kriz (28) that HMW kininogen is the preferred substrate for plasma kallikrein. These two observations are consistent with the predominant intravascular distribution of HMW kininogen but do not shed further light on the relationship between distribution and function of the kininogens.

Turnover of rhesus monkey kininogens was similar to that of human kininogens when measured in monkeys. This was to be expected in view of the similarities between the kininogens of the two species. However, Regoeczi has shown that in rabbits human fibrinogen disappeared at 1.45 times the rate of homologous fibrinogen before

immunologic clearance mechanisms were activated (29). Similar studies in monkeys injected with human fibrinogen showed its clearance rate to be 1.3 times faster than that of homologous fibrinogen despite the absence of any detectable immune response (22). The difference in clearance rates was attributable to differences in diffusion rates as well as in pool ratios. We did not observe any significant differences in plasma half-life between monkey and human kininogens, but the former had a slower catabolic rate than its human counterpart and this difference was apparently related to the former's significantly greater distribution into the intravascular pool. The differences in pool ratio between the heterologous and homologous LMW kininogens may reflect differences in their functional characteristics in monkeys.

Because of their unique and potent physiologic actions, the kinins have been implicated in the pathophysiology of a number of severe clinical illnesses. Evidence of kinin system activation in these illnesses, however, has been inferential for the most part because turnover of the proteins involved has not been evaluated. We have studied the syndrome of disseminated intravascular coagulation in Salmonella typhimurium infected rhesus monkeys and, as in other illnesses with suspected kinin system participation, our evidence of kinin activation has been limited to the indirect observations of elevated kallikrein and diminished prekallikrein and kininogen (30). We are presently attempting to clarify the association between kinins and clinical

illness by direct study of kininogen turnover in our infected monkeys and comparing with the normal studies presently discussed. In this fashion, we hope to be able to document the involvement of the kinin system in disseminated intravascular coagulation and to evaluate various potential therapeutic measures in relation to their effect on the kinin system. The technique for measuring kininogen turnover should also be applicable to the study of other illnesses in which the kinin system is thought to play an important pathophysiologic role.

ACKNOWLEDGEMENTS

The authors wish to express their appreciation to Dr. J. David Gangami, Mr. Edgar W. Larson, and Dr. William R. Beisel for their helpful criticism, Mrs. Phebe W. Summers for her editorial help, and Messieurs Glen A. Higbee and Dwayne D. Oland for their help with the computer analysis of our data. We gratefully acknowledge the technical assistance of Dr. Mocair De Sa Pereira, Messieurs Charles K. Ambrose, and Steven A. Tobery, and Ms. Cathleen Schmidt.

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TABLE I. Isolation procedure for plasma kininogens

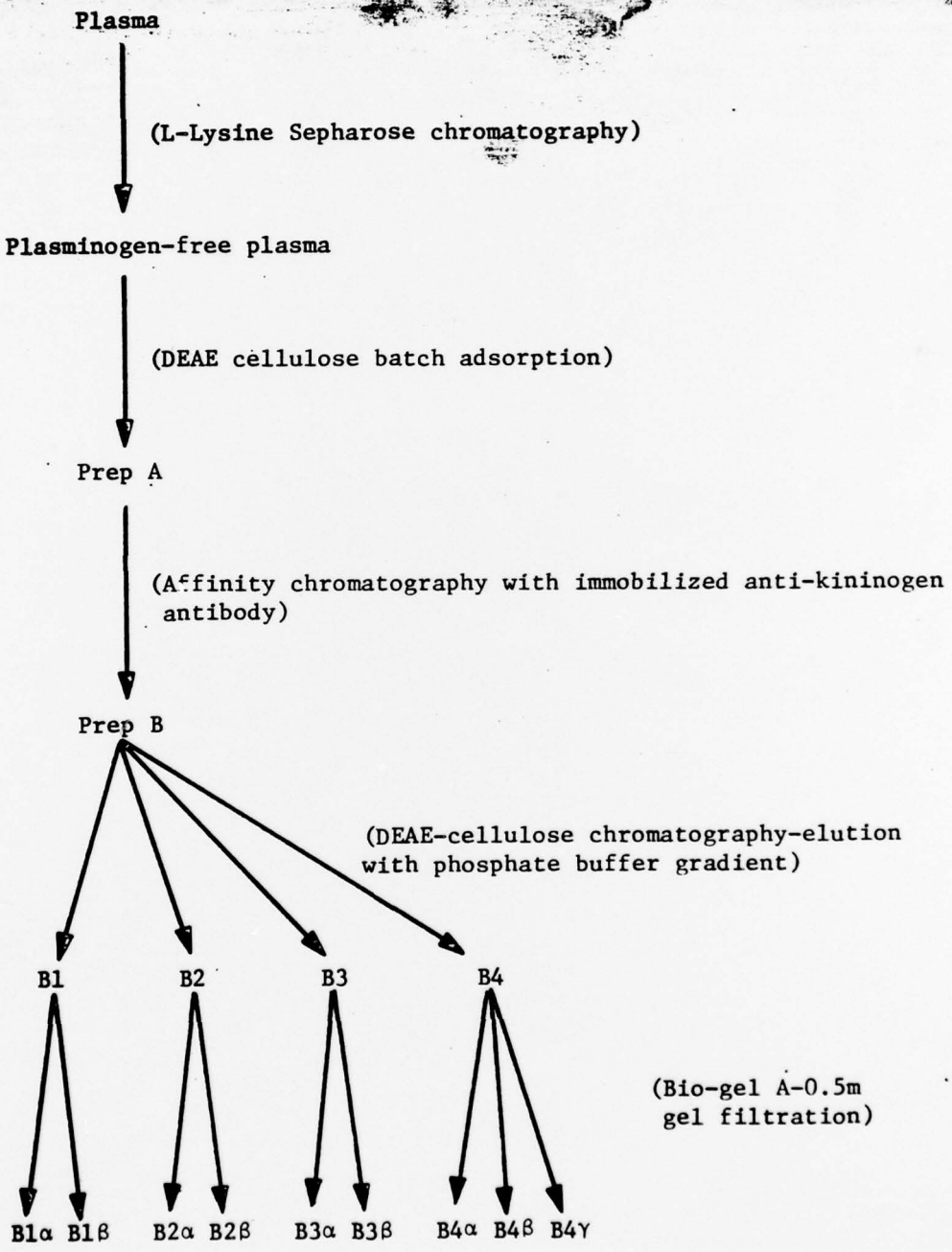


TABLE II. Comparison of kinetic parameters for disappearance of radiolabeled HMW versus LMW kininogens from plasma. The values given are the mean ± standard error for plasma half-life (T 1/2), mean catabolic rate (Fc), capillary transfer rate (Fi,e), return flow (Fe,i), and extravascular/intravascular pool ratio (ev/iv). Probabilities for any differences are noted on the bottom line unless insignificant (NS)

Kininogen	T 1/2	Fc	Fi,e	Fe,i	ev/iv
HMW	25.95±1.60	1.12±0.08	4.73±1.02	7.20±1.02	0.63±0.05
LMW	18.94±1.93	2.07±0.09	7.40±2.64	7.11±2.49	1.05±0.07
P	< 0.05	< 0.001	NS	NS	< 0.01

TABLE III. Sum of the constants ($C_1 + C_2$) of the two exponentials describing the disappearance of labeled kininogen from plasma

Study No.	PREP B $C_1 + C_2$	HMW Kininogen $C_1 + C_2$	LMW Kininogen $C_1 + C_2$
	%	%	%
1	99.2	97.5	99.6
2	98.5	97.6	99.8
3	100.0	99.9	100.0
4	99.9	100.0	99.9
5	100.0	100.0	
6	100.0	99.8	
Mean ± SD	99.6 ± 0.6	99.1 ± 1.2	99.6 ± 0.5

TABLE IV. Correlation coefficients (r) obtained by fitting disappearance of labeled kininogen to 2 versus 3 exponentials (exp.).

	Prep B		HMW Kininogen		LMW Kininogen	
Study No.	2 exp.	3 exp.	2 exp.	3 exp.	2 exp.	3 exp.
	r	r	r	r	r	r
1	0.9961	0.9963	0.9506	0.9915	0.9963	0.9967
2	0.9950	0.9951	0.9949	0.9953	0.9953	0.9962
3	0.9999	0.9999	0.9997	0.9998	0.9998	0.9998
4	0.9988	0.9998	0.9995	0.9996	0.9997	0.9998
5	0.9996	0.9999	0.9989	0.9989		
6	0.9996	0.9998	0.9985	0.9985		

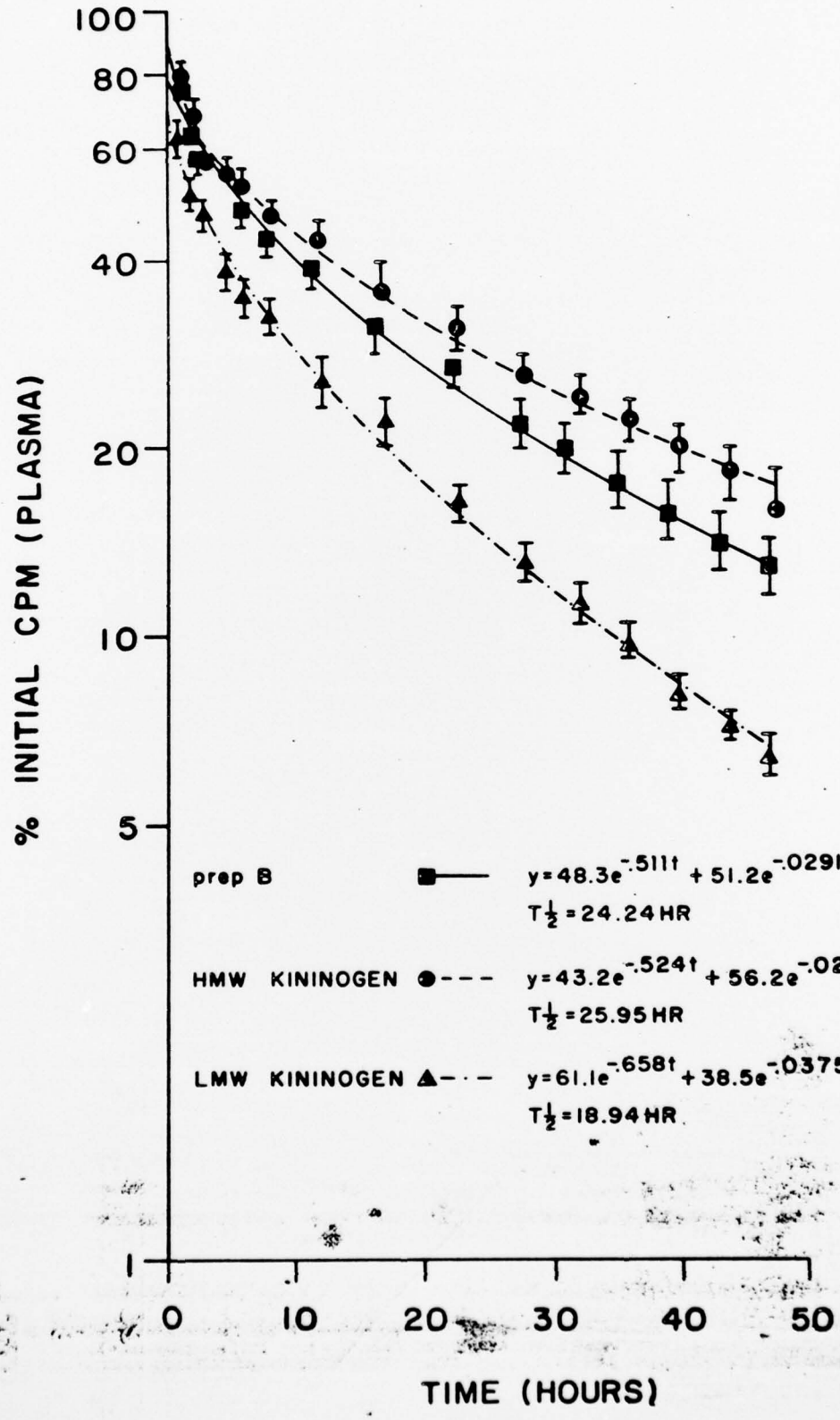
FIGURE LEGENDS

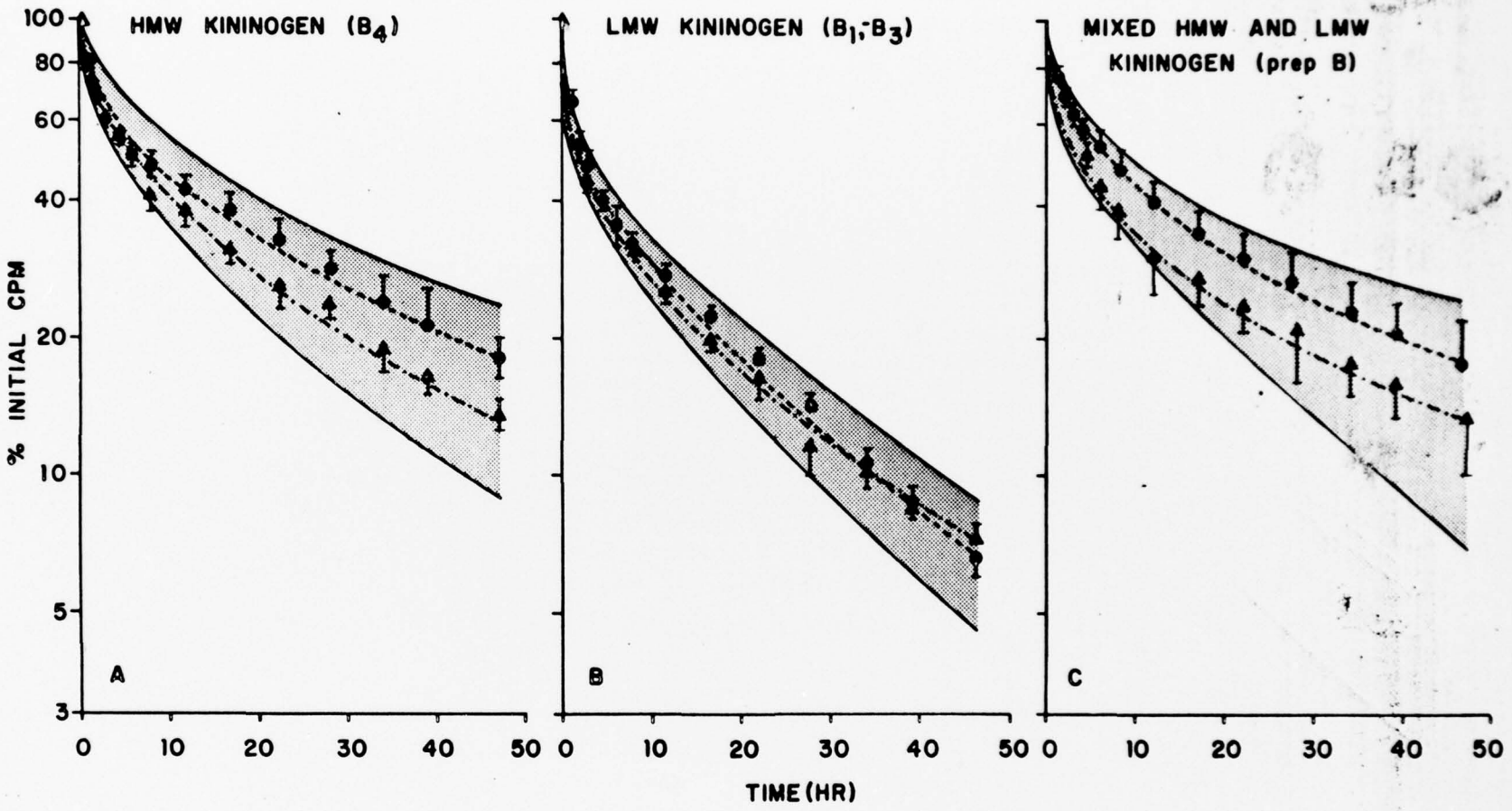
FIGURE 1. Turnover curves for human high molecular (HMW), low molecular weight (LMW), and mixed (prep B) kininogens. Radiolabeled proteins were administered intravenously to rhesus monkeys and their disappearance from the plasma on a log scale was plotted against time. The points represent mean ± standard error of 6, 4, and 6 studies for kininogens of high, low, and mixed molecular weight, respectively. The equations that best fit the data points and the plasma half-lives ($t_{1/2}$) for each of the kininogens are depicted at the bottom.

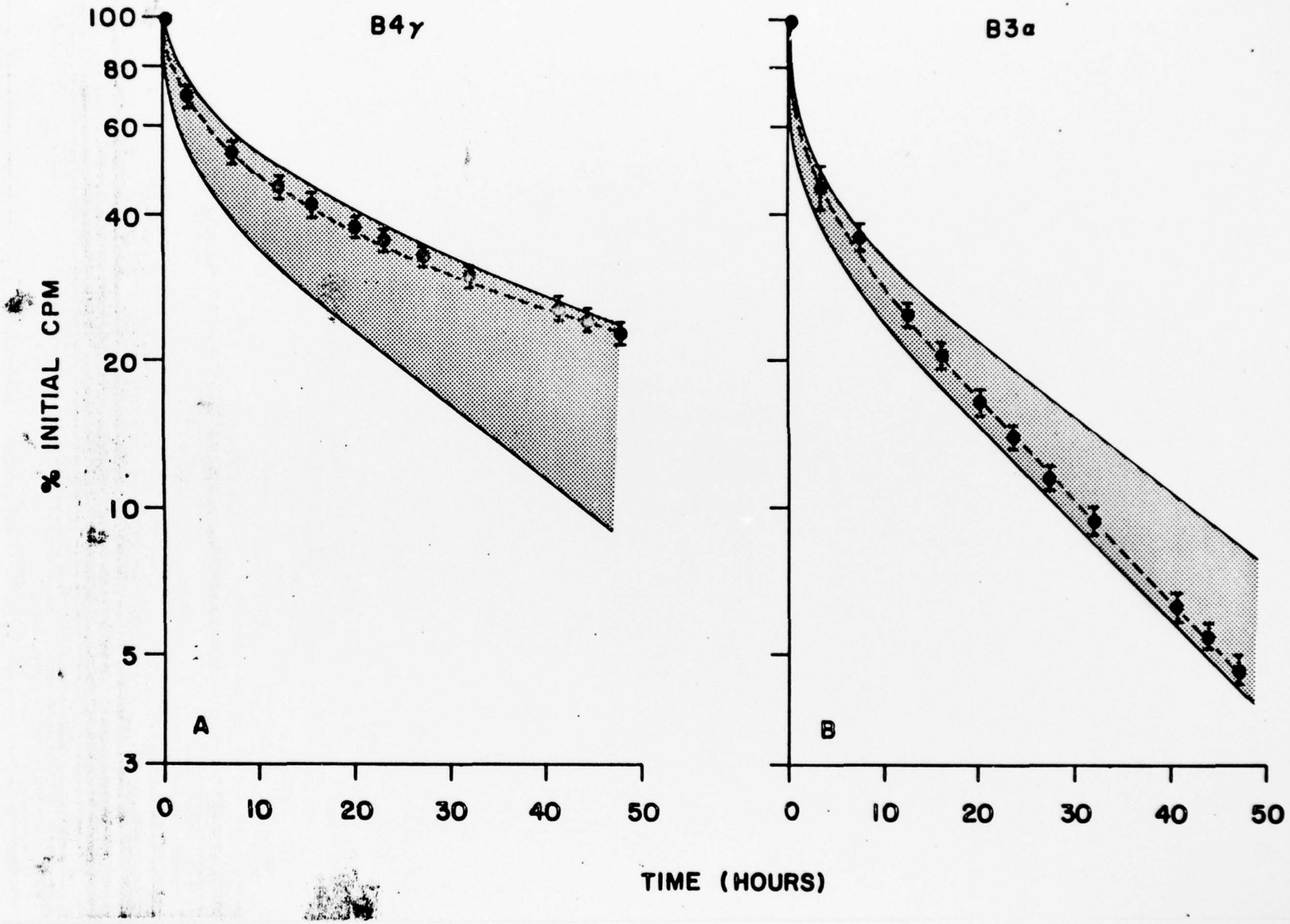
FIGURE 2. Comparison of turnover curves obtained by measuring whole blood and antibody-precipitated kininogen radioactivities against plasma radioactivity for HMW (A), LMW (B), and mixed kininogens (C). The 95% confidence limits for the line describing the disappearance of plasma radioactivity are represented by the shaded areas; the radioactivities for blood are depicted by triangles (▲) and kininogen precipitates by circles (●). Each point represents mean ± standard error for 4 studies.

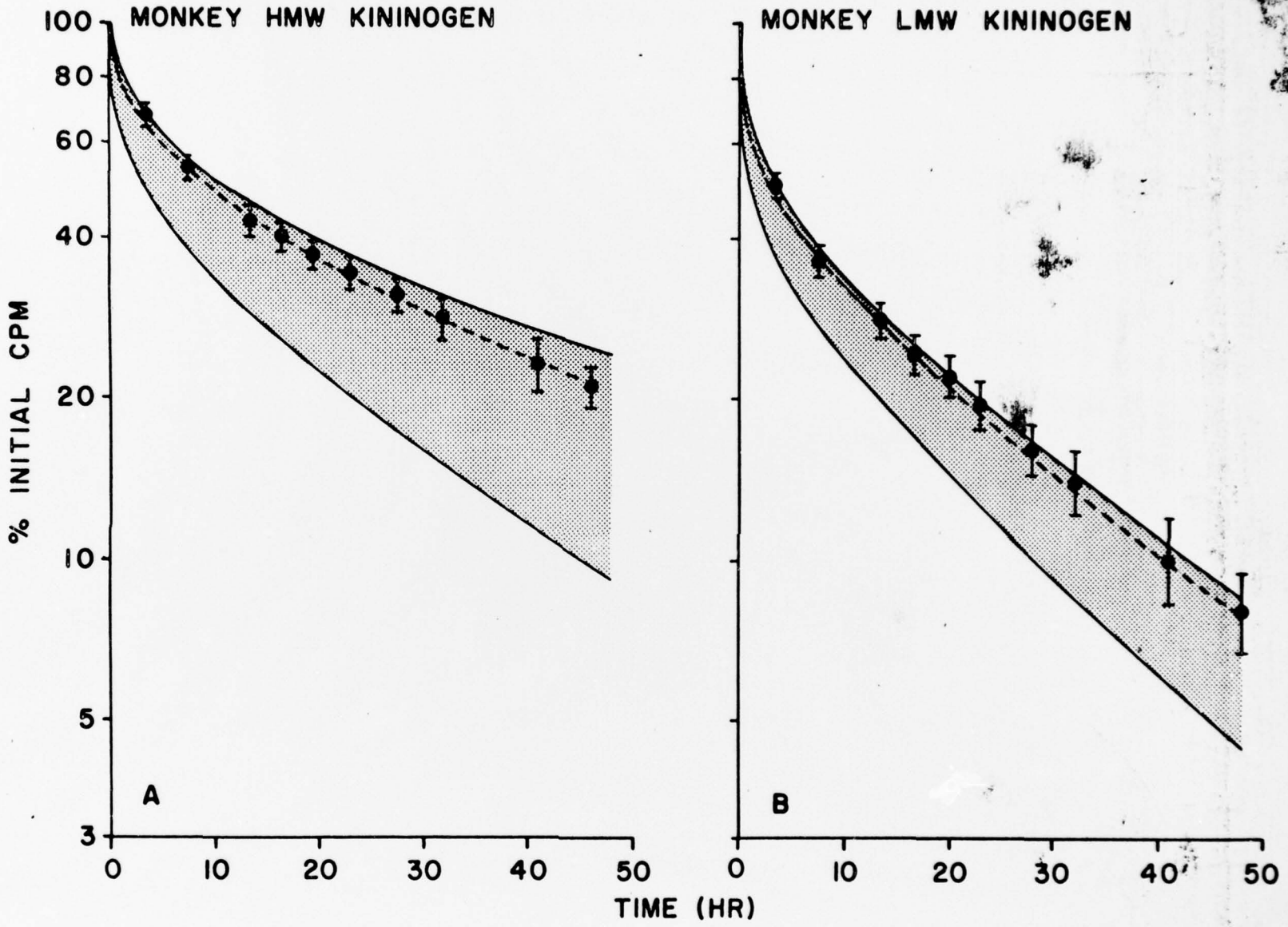
FIGURE 3. Comparison of disappearance curves for radiolabeled B4γ versus HMW kininogen (A) and B3α versus LMW kininogen (B). The 95% confidence limits for the turnover curves of HMW and LMW kininogen are represented by the shaded areas, and the circles (●) represent mean ± standard error of 4 studies each for B4γ and B3α.

FIGURE 4. Comparison of turnover curves for monkey HMW (A) and LMW (B) kininogens versus their corresponding human proteins. The 95% confidence limits for the turnover curves of human HMW and LMW kininogen are represented by the shaded areas, and the circles (●) represent mean ± standard error of 4 studies each for monkey HMW and LMW kininogen.


100
80
60
40
20
10
5
1
% INITIAL CPM (PLASMA)
0
10
20
30
40
50
TIME (HOURS)
prep B
y=48.3e^-.511t + 51.2e^-.0291t
T½ = 24.24 HR
HMW KININOGEN
y=43.2e^-.524t + 56.2e^-.0272t
T½ = 25.95 HR
LMW KININOGEN
y=61.1e^-.658t + 38.5e^-.0375t
T½ = 18.94 HR


HMW KININOGEN (B_4)
LMW KININOGEN (B_1-B_3)
MIXED HMW AND LMW KININOGEN (prep B)
% INITIAL CPM
100
80
60
40
20
10
5
3
A
B
C
0
10
20
30
40
50
TIME (HR)


B4γ
B3a
100
80
60
40
20
10
5
3
% INITIAL CPM
A
B
0
10
20
30
40
50
TIME (HOURS)


MONKEY HMW KININOGEN
MONKEY LMW KININOGEN
% INITIAL CPM
100
80
60
40
20
10
5
3
A
B
0
10
20
30
40
50
TIME (HR)